Protocol

Establishment of a Smoke Chamber for Exposure to Second-hand Smoke

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Received: 08 Jul 2023 | Revised: 15 Aug 2023 | Accepted: 09 Sep 2023 | Published Online: 13 Sep 2023
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Abstract

**Background:** With an ever-growing trend along with cheap and easy access to cigarettes, cigarette smoking has increased among people of all age groups from children to adults. Additionally, the number of people exposed to second-hand smoke (SHS) has also increased. Researchers are keenly studying the effects of smoking on the body of non-smokers exposed to SHS. Even though various smoke chambers have been constructed to date for this purpose, still the available literature does not suffice to describe in detail the development of such chambers. The present study aimed to construct a low-priced and simple smoke chamber that ensures effective SHS studies and enhances reproducibility.

**Material and methods:** The paper briefly described all the key steps and materials involved in the construction of the smoke chamber. The chamber was constructed using things commonly found in households and laboratories. To check the efficacy of this chamber, an experiment was conducted on mice to study the effect of SHS on their kidneys by exposing them to cigarette smoke for 7 consecutive days. After the designated time, all the mice were dissected, and pieces/parts of the kidney were collected, excised, and further processed for histological sectioning and staining. In the end, the histological results of the experimental mice group were compared with those of the control group.

**Results:** The histological results showed visible alteration in the microarchitecture of mice kidneys on chronic exposure to SHS.

**Conclusion:** Considering the experimental results obtained, it can be concluded well that the smoke chamber is highly effective in carrying out SHS-based experiments on mice. Furthermore, a detailed description of all steps may further help in the reconstruction of the smoke chamber in a short time thus allowing the successful replication of similar types of work.

**Key words:** Cigarette smoking; Environmental tobacco smoke; Second-hand smoke; Smoke Chamber

Introduction

The use of cigarettes among teenagers and adults for cigarette smoking adopted merely as a form of hobby, fun, fashion, or any other purpose mostly leads to long-term smoking addiction owing to nicotine dependence. No doubt with time the studies regarding the harmful effects of smoking have increased tremendously still there is an eminence increase in the number of smokers also due to cheap prices of cigarettes, easy availability for teenagers, and massive publicity by tobacco companies. The effects caused by exposure to cigarette smoke either in form of active or passive smoking on the body have been an utmost concern to researchers and healthcare workers.

Various studies have shown that smokers are not only putting their lives in danger but also putting the lives of others at great risk due to exposing them to second-hand smoke (Heffernan et al., 2016).
Active and passive smoking are considered serious global health hazards (Singh & Lal, 2011). To check the harmful effects of components of cigarettes on the human body, scientists and researchers have carried out various experiments and tests using many animal models for instance mice. To date, a number of smoking chambers have been constructed to expose mice to cigarette smoke. A lot of data is available on the effect of active smoking therefore most of the chambers that those studies discuss are only for exposure to active smoke. Comparatively, there is less data available on exposure to second-hand smoke and hence fewer such specially created chambers that allow exposure to second-hand smoke. This emphasizes the need for the development of a smoke chamber that allows exposure to second-hand smoke and helps in understanding its impacts on the body.

The aim of the present study was to develop a chamber that matches closely with the real conditions during exposure to second-hand smoke from a burning cigarette. The smoke chamber was constructed to simulate the natural environmental conditions created at the time of actual human smoking, keeping in view the aspects like the amount of cigarette smoke emanating from the burning ends of cigarettes and exposure to normal fresh air. Due to the wide availability of the materials involved, simple construction, and easy setup, the chamber is highly re-constructible. All the key steps involved in the construction of the chamber are discussed briefly for better adaption of the protocol by other researchers.

The objective of this protocol was to develop a brief, to-the-point, and step-by-step guideline for the construction of a smoke chamber to initiate further smoke studies.

**Experimental Design**

A smoke chamber was constructed for exposure to second-hand smoke using cheap and readily available materials. Details of all the materials and equipment used in the experiment are given below. The text also provides a detailed description of each step along with labeled pictures where needed. Upon the installation of the smoke chamber, an experiment was carried out to study its efficiency. Five female mice were kept in the newly constructed smoke chamber and exposed to cigarette smoke for seven consecutive days. While the other five female mice were kept in the control group in simple cages with exposure to normal fresh air. After the designated time, dissections were performed, and kidneys were removed and further processed for histological sectioning and staining. At last, the histological results of both groups viz experimental and control were compared and analyzed.

**Materials**

Acrylic plastic sheets (Pak Poly Industries, 0.5 cm thick); RTV Silicone sealant (GMSA); Plastic funnel (Local); Plastic marker (Master - Urdu marker # 604); Plastic bird cage feeder/ water bowl (Local); PVC tape (OSAKA); Cigarette (Any brand); Door Hinge (Local, 4 cm long).

**Equipment**

Three CPU Cooling fan (DELTA, Model: AFB0912SH, 12 Volt); AC/DC converter (APOLLO, Model: MS-1012, 1000mA); Digital thermometer (TITAN, HTC-1); Air quality detector (Guangzhou Guigao Electronics Co., Ltd., Model: PG-L58, 5V DC, 1200mAh).

**Procedure**

**Construction of smoke chamber**

1. Take 6 acrylic sheets of the following size:
   a. Two sheets 28×23 cm each for shorter sides of the rectangular box.
   b. Two sheets 38×23 cm each for the long sides of the rectangular box.
   c. Two sheets 38×28 cm each for the lower base and upper lid part of the box.

The overall prepared length of the box is 38 cm, the width of the box is 28 cm, and the height of the box is 23 cm. Figure 1 shows the diagrammatic representation of the smoke chamber along with its important components.

![Figure 1. Diagrammatic representation of smoke exposure chamber. Created in BioRender.com](image)

2. Now using a marker, mark some equally distanced points on one side of the long (38 cm× 23 cm) acrylic sheet as shown in Figure 2. Then carefully use a drilling machine to make holes of 0.8cm in the marked points on the acrylic sheets. Make four rows of holes in it. Repeat the process on another same-sized sheet. These holes will be used for the purpose of ventilation allowing fresh air to enter into the smoke chamber.

![Figure 2. Diagrammatic representation of dimensions of the acrylic sheet.](image)
3. Take a 38 cm × 28 cm sized sheet and cut an 18 cm × 23 cm part in it. Take the cut-out part and again fix it to the same place using door hinges. This acts as a lid that serves the purpose of feeding and transferring mice, cleaning the cages, and manually igniting the cigarette. In the remaining part drill, more holes using a drilling machine.

![Figure 3. Actual photograph of the upper sheet of the chamber representing the lid and drilled holes along with its measurements.](image)

4. Now one by one apply silicone sealant on the edges of each sheet and join them together. Start by attaching the longer sheets on the lower base sheet of the box (Figure 4A, 4B).

![Figure 4. Joining acrylic sheets. A) RTV silicone sealant used for joining. B) Two acrylic sheets joined on the base sheet of the chamber.](image)

5. Then join the other sides and lastly join the top lid sheet (Figure 5A-C). Allow the sealant to dry completely before joining the next sheet.

6. When all sheets join successfully, apply more sealant on joined edges to make them stronger and waterproof.

**Setting up cigarette holder and ashtray**

7. Take a cap of a plastic marker and cut its close end. This cap will act as a cigarette and funnel holder (Figure 6).

![Figure 6. Flowchart of the process of making the cigarette holder.](image)

8. Now fix this in a suitable hole in any of the side sheet (left or right) in such a way that half of the holder remain inside the chamber and remaining half lies outside the chamber. For instance, if the cap is 4 cm long, keep 2 cm of the cap inside the chamber and the
remaining 1-2 cm of the cap outside the chamber (Figure 7A, 7B).

9. Check the holder by placing a cigarette in it so the butt end of the cigarette completely goes inside while the remaining part remains outside and stays vertical perfectly. Using a metal wire, fix a bird-feeding plastic bowl below it to act as an ashtray (Figure 8). Make sure the ashtray is not in the reach of the mice.

3.3. Setting up an electric fan

To simulate the natural puffing phenomenon, an electric fan is attached with help of a plastic funnel to the cigarette holder to suck air from the cigarette and keep it burning. The required materials are shown in Figure 9. Buy the funnel according to the size of the fan. Use metal wires to fix the fan into the funnel.

10. Now fix the cooling fan into the plastic funnel as shown in Figure 10. Cover the narrow funnel stem to elongate it by forming a small flexible tube using PVC tape to easily attach it to the cigarette holder.

11. Connect the fan to an AC/DC converter (Figure 11) for controlling the speed of the fan thus maintaining the total time of burning of a cigarette.

Assembling parts

12. Then carefully fix the funnel stem into the remaining back end of the marker outside the chamber (Figure 12). Give support to the funnel and fan to keep them in the desired horizontal position.

Handling and monitoring

13. Attach a water bottle to the chamber by entering the nozzle through a hole in the acrylic sheet. Put the mice feed into the chamber by opening the chamber lid present on top. Make sure to clean the chamber on daily basis, take out the ash from the ashtray, and refill the water bottles.
Establishment of a smoke chamber for exposure to second hand smoke

14. Place the multifunctional air quality detector inside the chamber. This allows accurate real-time monitoring of humidity, temperature, amounts of carbon dioxide (CO2), formaldehyde (HCHO), and volatile organic compounds (TVOC) (Figure 13). To double-check, the temperature measurements fix a digital thermometer (TITAN, HTC-1) on the upper side of the chamber and hang its probe into the chamber by entering it from any side hole made for ventilation purposes. This allows real-time observation and recording of the changes in temperature with greater accuracy.

![Figure 12. The final assemblage of the smoke chamber.](image)

![Figure 13: Real-time monitoring A) Air quality detector fixed inside the smoke chamber. B) Digital thermometer fixed on the upper sheet of the chamber.](image)

Connecting to the power supply and setting up the voltage

15. Now connect the AC/DC converter to the power supply. Also, make sure that all the spliced wire connections are nicely wrapped with electrical tape.

16. **▲ CRITICAL STEP** Carefully select the voltage level on AC/DC converter as this controls the speed of the electric fan and therefore controls the burning timing or speed of burning of a single cigarette. By setting a low voltage level, fan rotation slows down, and consequently the cigarette burns slowly and takes more time to burn, and vice versa. Before putting the mice into the smoke chamber, run a trial for adjusting the desired burning time by burning a cigarette. The chamber is now fully functional and ready to use.

**Operating smoke chamber**

17. To operate the chamber, open the lid cover and put a cigarette into the holder. Then turn ON the power supply. The fan will start rotating and sucking air. Now light the cigarette and close the lid. Wait for the cigarette to burn. Now turn OFF the power supply and pull out the cigarette butt using forceps. Empty the ashtray and attach it back.

**Results**

The use of this chamber helped in studying the effect of SHS on the kidneys of mice very effectively. Significant results were obtained for histologically stained slides of mice kidneys with evident changes in the microarchitecture of mice kidneys. This proves the effective use of this smoke chamber in cigarette smoke-related studies.

The manual building of the chamber allows for choosing and adjusting the size of the chamber according to the number of animals to be kept inside it for smoke exposure. Pre-build smoke machines do not allow one to make such flexible changes according to the layout of the experiment. Also, further partitions could be made in the chamber using acrylic sheets to study the effect of cigarettes on both genders simultaneously or to study the effect on different kinds of mice, or with different health conditions.

Additionally, the chamber allows to manually light the cigarette so as many cigarettes can be burnt as required, whereas automated smoke machines have only a fixed number of cigarettes to be filled and burn per given time. Another benefit is that the manual lighting of cigarettes provides variable gaps between each cigarette and different timings for the complete burning of a cigarette owing to its dependence on the electrical supply. This is closer to the natural conditions in which a person takes different gaps between cigarettes and has varying smoldering patterns. Automated smoke machines mostly lack such features and work on a fixed time for each cycle.

Furthermore, the chamber allows wide space for mice to roam and feed, unlike some machines that allow less or congested space, which makes the mice more stressed and might affect the actual results. The use of low-price carbon dioxide monitors has made the monitoring of gasses very cheap and allows real-time monitoring. Moreover, the overall handling of the chamber is very easy and needs no expertise, unlike other complex smoke machines that require staff training. Once the chamber is set up, it needs no further changes, it is easy to use. It can be constructed within a few hours. No need to order, high-priced smoke machines and wait for their delivery. On top of that, all the materials and equipment used in the construction are cheap, reliable, and easily available.

Taken together, we highly recommend the use of this chamber for setting up experiments on mice for studying the effect of SHS on behavior, weight, diet preferences, organs, etc.

**Conclusion**

To the best of our knowledge, this protocol provides for the first time a practical approach for the construction of a smoke chamber in any ordinary laboratory setting by using cheap and easily available resources for the rapid study of the effects of SHS on the body. As the chamber construction is manual it
provides the ability to freely choose the size of the chamber according to desired requirements. In addition to this, the results obtained from the SHS exposure experiment on mice in the self-build smoke chamber emphasize greatly the detrimental effects caused on passive smokers by the notorious smoking habit of smokers. It also stimulates the need for similar studies to be carried out on different organs and organ systems of mice using this smoke chamber so that the results could be generalized and applied to humans.

Acknowledgment
Authors are grateful for vice chancellor of University of Okara for providing support for this study.

References